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National Food Institute
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Validation Report 23 B

Determination of pesticide residues in wheat, rye, oat and rice by LC-MS/MS and GC-MS/MS

(QuEChERS method)

Susan Strange Herrmann
Mette Erecius Poulsen
January 2018

CONTENT:

1. Introduction

This report describes the validation of the QuEChERS method combined with GC-MS/MS and LC-MS/MS. The method was tried validated for 20 pesticides and metabolites by both LC-MSMS and GC-MSMS in wheat, rye, oat and rice. The QuEChERS method is an extraction method which has been developed to be Quick, Easy, Cheap, Efficient, Rugged and Safe. The method is most commonly used on fruit, vegetables and cereals[1].

2. Principle of analysis

Sample preparation: The samples is milled with a sieve at 1 mm.

Extraction: The sample is shaken and a salt and buffer mixture is added and the sample is shaken again.

Clean-up: After centrifugation the supernatant is transferred to a clean tube and put in -80 degree freezer. When the extract is almost thawed it is centrifuged and the supernatant is transferred to a tube containing PSA and $MgSO_4$. An aliquot was withdrawn prior to this clean-up step and analysed by LC-MS/MS. After shaking and an additional centrifugation step the final extract is diluted 1:1 with acetonitrile to obtain the same matrix concentration as in the matrix matched calibration standards.

Quantification and qualification: The final extracts are analysed by GC-MS/MS. Crude extract withdrawn before PSA clean-up was analysed by LC-MS/MS.

GC-MS/MS: The pesticide residues were separated on a DB5-MS column and analysed by triple quadrupole operating in the multiple reaction monitoring mode (MRM) with electron energy at 70 eV, source temperature at 180°C and transfer line at 250°C. The injection volume was 1 µl. For each pesticide minimum two sets of precursor and product ions were determined. One for quantification and one for qualification. The MRM transitions for the pesticides and degradation products are given in Appendix 1a.

LC-MS/MS: The pesticide residues are separated on a reversed-phase column and detected by tandem mass spectrometry (MS/MS) by electrospray (ESI). The validation includes pesticides determined in positive mode. All pesticides were detected in the MRM mode. For each pesticide or metabolite a precursor ion and 2 product ions were determined. One product ion for quantification and one for qualification. The MRM transitions for the pesticides and degradation products sought validated are given in Appendix 1b.

3. Validation design

The method was sought validated for almost 20 pesticides or metabolites in wheat, see **Appendix 1**. The validation was performed on 5-6 replicates on wheat at each of the three spiking levels; 0.005, 0.01 and 0.05 mg/kg. A blank sample of each cereal commodity was included.

4. Chromatograms and calibration curves

The calibration curve is determined by the analysis of each of the analysts at least 4 calibration levels within the range of 0.03, 1, 3.3, 10, 33.3 and 100 ng/ml. The calibration curves were in generally best fitted to a linear curve. The quantification was performed from the mean of two bracketing calibration curves. The majority of the correlation coefficients (R) were higher or equal to 0.99.

5. Validation parameters

Precision – repeatability and internal reproducibility

Repeatability was calculated for all pesticides and degradation products on all three spiking levels (0.005 mg/kg, 0.01 mg/kg and 0.05 mg/kg). Repeatability is given as the relative standard deviation on the result from two or more analysis at the same sample, done by the same technician, on the same instrument and within a short period of time.

Repeatability (RSD_r) in this validation was calculated from the 5-6 replicate determinations. Repeatability were calculated as given in ISO 5725-2[2].

Accuracy – Recovery

The accuracy was determined from recovery studies in which samples were spiked at three concentration levels (0.005 mg/kg, 0.01 mg/kg and 0.05 mg/kg) with the relevant pesticides, isomers and degradation products.

Robustness

The QuEChERS method has, in connection with the development of the method, been shown to be robust by Anastassiades et al. 2003[1].

Limit of quantification, LOQ

The quantification limits (LOQ) was determined as the lowest spike level for which the acceptance criteria (se Section 6) was meet.

The obtained results including recovery, RSD_r, RSD_R, Combined Uncertainty (U_c) and limit of quantification (LOQ) are presented in appendix 2.

6. Criteria for the acceptance of validation results

For the pesticides to be accepted as validated the following criteria for precision and trueness must to be fulfilled:

1. The relative standard deviation of the repeatability should be ≤20%[3].
2. The average relative recovery must be between 70 and 120%[3].

If the above mentioned criteria have been meet, the quantification limits, LOQs is stated.

The analytical result is by default corrected for bias/recovery and the combined uncertainty is then given by:

$$U_c = \sqrt{(RSD^2/n) + RSD^2}$$

Where RSD is the intra-laboratory uncertainty (RSD_R).

7. Results and conclusion

The validation results obtained for the 20 pesticides or metabolites using LC-MSMS and GC-MSMS are presented in appendix 2. For 18 compounds an LOQ of 0.005 mg/kg was achieved and for the remaining two compounds an LOQ of 0.01 and 0.05 mg/kg was achieved.

9. References

1 EN 15662:2008. Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method

2 ISO 5725-2:1994. Accuracy (trueness and precision) of measurement methods and results – Part2. Basic method for the determination of repeatability and reproducibility of standard measurement method. First edition. December 1994.

3 Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed, Document SANTE/11813/2017, 21–22 November 2017 rev.0, European Commission, Brussels, 2017.

Appendix 1a. MRM transitions for GC-MS/MS for compounds in mixture H.

Pesticide name	RT	Precusor Mass	Product Mass	Collision Energy
1,4-dimethylnaphthalene	9.63	115.1	89.1	15
1,4-dimethylnaphthalene	9.63	141.1	115.1	15
1,4-dimethylnaphthalene	9.63	156.1	141.1	15
Benzovindiflupyr	29.15	239	174	25
Benzovindiflupyr	29.15	369.1	159.1	20
Benzovindiflupyr	29.15	369.1	237.1	10
Bixafen	28.6	159	139.1	10
Bixafen	28.6	160.1	140.1	10
Bixafen	28.6	413.1	159.1	10
Fluensulfone	10.82	108	88	10
Fluensulfone	10.82	119	92	10
Fluensulfone	10.82	226	206	15
Flumetralin	17.74	143	107	25
Flumetralin	17.74	143	108	25
Flumetralin	17.74	143	142.2	30
Flumetralin	17.74	157	129	20
Flupyradifurone	7.47	156.1	155.1	15
Flupyradifurone	7.47	206.1	156.1	15
Flupyradifurone	7.47	275.1	206.1	15
Ipconazole	24.92	125	89.1	15
Ipconazole	24.92	249.1	125	20
Isoxaflutole	15.89	160	132	10
Isoxaflutole	15.89	189	161.1	10
Isoxaflutole	15.89	279	252	10
Mandestrobin	22.47	160.1	91.1	20
Mandestrobin	22.47	160.1	119.1	5
Mandestrobin	22.47	192.1	160.1	5
Pentachloraniline	13.98	264.9	193.9	20
Pentachloraniline	13.98	266.9	194	20
Quintozene	12.72	142	107	25
Quintozene	12.72	213.9	179	10
Quintozene	12.72	294.9	236.9	15
Sedaxane	25.23	130.1	77.1	25
Sedaxane	25.23	159.1	139	10
Sedaxane	25.23	172.1	130.1	10
Valifenalate	24.33	116.1	98.1	5
Valifenalate	24.33	155.1	139	20
Valifenalate	24.33	158.1	98.1	10

Appendix 1b. MRM transitions for LC-MS/MS for compounds in mixture H.

LC-MS/MS	Mode	Retention time	Precursor ion-1	Product ion-1	CE	Precursor ion-2	Product ion-2	CE
Acequinocyl	Positive	8.91	402	343.2	-12	402	189	-35
Benzovindiflupyr	Negative	6.28	397.2	369.6	19	397.2	43	43
Bixafen	Positive	6.15	414	394	-15	414	266	-25
Chlorantraniliprole	Positive	4.93	484.3	285	-15	484	453	-15
Chlorfluazuron	Positive	7.71	540	158	-17	540	383	-19.5
Flubendiamide	Negative	6.15	681.4	254.2	25	681.4	274.3	13
Haloxyfop	Positive	6.36	362	316	-12	360	288	-12
Isoxaflutole	Positive	4.66	360	251	-16	360	220	-37
Mandestrobin	Positive	6.28	314.4	192	-7	314.4	160	-17
Oxasulfuron	Negative	3.53	405.4	182.2	9	405.4	122.3	20
Sedaxane	Positive	5.63	333	159	-16	333	292	-11.5
Sulfoxaflor	Positive	2.81	278.3	174	-8	278.3	154	-25
Valifenalate	Positive	5.52	399	116	-20	399	155	-31

Appendix 2. Recoveries, repeatability (RSD_r), internal reproducibility (RSDR) and Limit of Quantification (LOQ) for pesticides validated on three cereal commodities, oat, rye and wheat using QuEChERS.

Numbers marked with a colour is outside 70-120% recovery (red) or above 20% RSD (orange)

		Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.1 mg/kg				
	Compound	Recovery %	RSD_r, %	RSDR, %	Comb. Uncer-tainty (%)	Recovery %	RSD_r, %	RSDR, %	Comb. Uncer-tainty (%)	Recovery %	RSDr, %	RSDR, %	Comb. Uncer-tainty (%)	LOQ
GC	1,4-dimethylnaphthalene	105	17	17	18	81	14	*22*	*24*	94	15	18	16	0.01
LC	Acequinocyl									99	15	20	20	0.05
LC	Benzovindiflupyr					86	18	*26*	*26*	94	14	*22*	*22*	0.05
GC	Benzovindiflupyr	97	9	11	10	89	9	10	14	94	6	7	9	0.005
LC	Bixafen	102	10	14	15	98	13	14	14	102	7	9	10	0.005
GC	Bixafen	98	8	11	8	90	9	13	14	94	5	6	7	0.005
LC	Chlorantraniliprole	100	12	12	13	95	12	17	17	95	9	15	16	0.005
LC	Chlorfluazuron	79	16	16	16	75	18	18	19	78	14	*23*	*24*	0.005
LC	Flubendiamide	100	15	15	16	98	13	15	16	104	10	11	11	0.005
GC	Fluensulfone	97	16	*21*	*21*	87	15	20	20	99	6	6	6	0.005
GC	Flumetralin	97	10	12	13	74	21	*29*	*30*	79	12	12	13	0.005
GC	Flupyradifurone	113	8	16	16	105	8	20	20	113	12	14	14	0.005
LC	Haloxyfop	101	10	13	13	98	12	15	16	102	6	9	10	0.005
GC	Ipconazole	93	11	18	19	82	15	19	20	90	8	8	8	0.005
LC	Isoxaflutole	117	15	17	17	107	13	20	*21*	106	13	16	16	0.005
GC	Isoxaflutole	89	23	*28*	*28*	76	16	16	16	83	11	15	15	0.01
LC	Mandestrobin	96	10	13	14	94	11	13	13	101	6	10	10	0.005
GC	Mandestrobin	96	11	11	11	85	11	12	12	89	6	9	9	0.005
LC	Oxasulfuron	94	12	14	14	93	13	14	14	96	6	7	7	0.005
GC	Pentachloraniline	84	17	17	18	77	9	18	18	89	6	9	9	0.005
GC	Quintozene	94	18	18	19	70	19	*36*	*37*	101	13	17	18	0.005
LC	Sedaxane	100	14	13	13	94	12	15	16	100	5	9	9	0.005

		Spike level 0.005 mg/kg				Spike level 0.01 mg/kg				Spike level 0.1 mg/kg				
	Compound	**Recovery %**	**RSD_r, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**Recovery %**	**RSD_r, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**Recovery %**	**RSDr, %**	**RSDR, %**	**Comb. Uncer-tainty (%)**	**LOQ**
GC	Sedaxane	96	10	10	11	85	8	8	8	90	5	8	8	0.005
LC	Sulfoxaflor	105	12	19	19	104	9	9	9	105	8	10	11	0.005
LC	Valifenalate	105	11	13	13	96	14	14	14	98	12	15	15	0.005
GC	Valifenalate	100	8	10	10	91	9	10	10	95	6	7	7	0.005

Appendix 3: Principles of the QuEChERS method for cereal extraction

QuEChERS for cereals (FP417)

Weigh 5 g (±0.05 g) of flour into a 50 ml single use centrifuge tube (red cap). Add internal standard and/or spike standard (maximum 25 µl)

Add a ceramic homogenizer and 10 g of cold water and shake briefly

Add 10 ml acetonitrile and shake vigorously by hand for 1 min. (1. extraction)

Add the prepared mixture of 4 g $MgSO_4$, 1 g NaCl, 1 g Na_3 citrate dihydrate and 0.5 g Na_2H cirate sesquihydrate. Shake for a few seconds after each addition to prevent lumps.

Shake vigorously for 1 min. (2. Extraction with phase separation)

Centrifuge for 10 min at 4500 rpm

Transfer at least 8 ml of the extract to a 15 ml single use centrifuge tube and store in the freezer (-80˚C for 1 hour or over night). When the extract are almost thawed (i.e. About -40 ˚C) centrifugate (should be cold 5 C) for 5 min. at 4500 rpm.

Transfer 6 ml of the cold extract to a 15 ml single use centrifuge tube containing 150 mg PSA and 900 mg $MgSO_4$. Close the tube and shake vigorously for 30 seconds.

Centrifuge for 5 min. at 4500 rpm

Transfer 4 ml of the extract to a 15 ml single use centrifuge tube. Add 40 µl of 5% formic acid solution in acetonitrile (10 µl/ml extract). Dilute the extract 1:1 with acetonitrile

Transfer the final extract into auto sampler vials and analyse by GC and LC.